PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION See Form PCT/IPEA/416	
PC-21007191		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/SE 2003/001949	12.12.2003	13.12.2002
International Patent Classification (IPC) or national classification and IPC		
A61K39/395, A61K47/48, A61K51/10 // A61M1/36		
Applicant		
Mitra Medical Technology AB et al		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *(sent to the applicant and to the International Bureau)* a total of 6 sheets, as follows:
      - ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      - ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.

   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s))
      _____, containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:
   - ☒ Box No. I Basis of the report
   - ☐ Box No. II Priority
   - ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☐ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☐ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☒ Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
05.07.2004	11.04.2005
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Form PCT/IPEA/409 (cover sheet) (January 2004)

CORRECTED

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/SE 2003/001949

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ This report is based on a translation from the original language into the following language _____, which is the language of a translation furnished for the purposes of:

☐ international search (under Rules 12.3 and 23.1(b))
☐ publication of the international application (under Rule 12.4)
☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report):*

☐ the international application as originally filed/furnished

☒ the description:

pages 1-50 as originally filed/furnished
pages* _____ received by this Authority on _____
pages* _____ received by this Authority on _____

☒ the claims:

pages _____ as originally filed/furnished
pages* _____ as amended (together with any statement) under Article 19
pages* 1-6 received by this Authority on 22.03.2005
pages* _____ received by this Authority on _____

☒ the drawings:

pages 1-6 as originally filed/furnished
pages* _____ received by this Authority on _____
pages* _____ received by this Authority on _____

☐ a sequence listing and/or any related table(s) – see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____
☐ the claims, Nos. _____
☐ the drawings, sheets/figs _____
☐ the sequence listing *(specify)*: _____
☐ any table(s) related to the sequence listing *(specify)*: _____

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

☐ the description, pages _____
☐ the claims, Nos. _____
☐ the drawings, sheets/figs _____
☐ the sequence listing *(specify)*: _____
☐ any table(s) related to the sequence listing *(specify)*: _____

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/SE 2003/001949

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application

☒ claims Nos. 21

because:

☒ the said international application, or the said claims Nos. 21 relate to the following subject matter which does not require an international preliminary examination *(specify)*:

See PCT Rule 67.1.(iv).: Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. _____ are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for said claims Nos. _____

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form
- ☐ has not been furnished
- ☐ does not comply with the standard

the computer readable form
- ☐ has not been furnished
- ☐ does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in the Annex C-*bis* of the Administrative Instructions.

☐ See Supplemental Box for further details.

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/SE 2003/001949

Box No. V **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-20	YES
	Claims		NO
Inventive step (IS)	Claims	1-20	YES
	Claims		NO
Industrial applicability (IA)	Claims	1-20	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

The claimed invention pertains to a medical agent comprising a reagent conjugated to an anti-CD20 antibody.

Reference is made to the following relevant documents:

D1: WO 0002050
D2: US 2001023288
D3: WO 0009160
D4: WO 0180884

Documents D1 and D2 describe a trifunctional reagent for conjugation to a biomolecule for diagnosis and treatment of human or animal conditions and diseases. The reagent comprises a trifunctional cross-linking moiety coupled to an affinity ligand, to an effector agent and to a biomolecule reactive moity. The affinity ligand may for example be biotin or a biotin derivative, the effector agent may for example be a toxin, an immunosuppressive agent or a radionuclide and the biomolecule reactive moiety is capable of forming a bond between the reagent and a biomolecule.

According to documents D1 and D2 the reagent may be conjugated to a biomolecule and used in a method for diagnosis or treatment. It can be administered to the blood circulation of a mammal in order to be concentrated to the target tissue or the cells and it is also possible to remove the biomolecules which are not attached to the target tissue from the blood circulation, through using the affinity ligand. It is further disclosed in documents D1 and D2 that monoclonal antibodies

.../...

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Box V

capable of binding cancer cells are used in the treatment of cancer, then being conjugated to various toxins and radionuclides. It is also suggested in the claims that the reagent described in documents D1 and D2 could be used in the treatment of cancer.

The medical agent according to the invention differs from what is described in documents D1 and D2 in that the biomolecule is conjugated to 1.5 to 3.5 reagents and in that the biomolecule is an anti-CD20 antibody. The problem which has been solved in the present application in relation to D1 and D2 is that by coupling the antibody to 1.5 to 3.5 regents the conjugate is able to bind with high selectivity and high affinity to cells expressing CD antigens. In this way higher doses can be given to a patient without severe effects on sensitive tissues like the bone marrow.

D3 and D4 discose the use of anti-lymphoma antibodies, such as anti-CD20 antibodies (e.g.rituximab), conjugated to radioisotopes or toxins.

It is considered unobvious to a person skilled in the art to prepare a conjugate of an anti-CD20 antibody with 1.5 to 3.5 reagents according to the claimed invention and that it still retains its binding profile both in respect to antigen selectivity and receptor affinity.

Therefore the claims are novel and are considered to involve an inventive step.

CLAIMS

1. A medical agent comprising an anti-CD20 antibody or variants thereof conjugated to 1.5 to 3.5 reagents, wherein each reagent comprises

a) a trifunctional cross-linking moiety selected from the group consisting of triaminobenzene, tricarboxybenzene, dicarboxyaniline and diaminobenzoic acid, coupled to

b) a biotin molecule selected from the group consisting of biotin and biotin derivatives having essentially the same binding function to avidin or streptavidin as biotin, via a linker 1, wherein the linker 1 contains hydrogen bonding atoms, preferably ethers or thioethers, or ionisable groups, preferably carboxylate, sulphonates and ammonium to aid in water solubilisation of the biotin moiety, and stability against enzymatic cleavage has been provided by introducing substituents to the biotinamide amine or to an atom adjacent to that amine, to

c) an effector agent covalently linked to the trifunctional cross-linking moiety, optionally via a linker 2, wherein the linker 2 provides a spacer length of 1-25 atoms and the linker contains hydrogen bonding atoms, preferably ethers or thioethers, or ionisable groups to aid in water solubility, and to

d) a linker 3, which covalently links the anti-CD20 antibody to the reagent, wherein the linker 3 provides a spacer length of 1-25 atoms and contains hydrogen bonding atoms, preferably ethers or thioethers, or ionisable groups to aid in water solubility, wherein the anti-CD20 antibody is selected from a group of antibodies or

variants thereof having a specific binding to CD20 antigens and having an affinity binding constant of at least $5x10^6$ M^{-1}.

2. The medical agent according to claim 1, wherein the anti-CD20 antibody is conjugated with from 3 to 4 reagents.

3. The medical agent according to any one of the preceding claims, wherein the affinity binding constant is at least 10^8 M^{-1}.

4. The medical agent according to any one of the preceding claims, wherein the anti-CD20 antibody is ibritumomab, rituximab, or tositumomab.

5. The medical agent according to claim 4, wherein the anti-CD20 antibody is rituximab.

6. The medical agent according to any one of the preceding claims, wherein the linkers 2 and 3 provide a spacer length of 6-18 atoms.

7. The medical agent according to any one of the preceding claims, wherein the anti-CD20 antibody variant has the same or essentially the same ability as the anti-CD20 antibody to bind to both the anti-CD20 antibody reacting moiety and said CD antigen/antigens on the surface of a lymphoma tumour cells, and wherein said variant is an antibody derivative, preferably the F $(ab')_2$, F (ab') or F (ab) fragment, genetically engineered hybrids or chemically synthesized peptides, preferably chimeric or humanized antibodies, and single chain antibodies.

8. The medical agent according to any one of the preceding claims, wherein the effector agent is a radionuclide bidning moiety, optionally provided with a radionuclide, a synthetic or naturally occurring toxin, an enzyme capable of converting pro-drugs, immunosuppres-

sive or immunostimulating agents, radiosensitizers, enhancers for X-ray of MRI or ultrasound, non-radioactive elements, which can be converted to radioactive elements by means of external irradiation after the anti-CD20 antibody carrying said element has been accumulated to specific cells or tissues, or photoactive compounds or compounds used in photo-imaging or photodynamic therapy, or any other molecule having the same or similar effect, directly or indirectly, on lymphoma cells or lymphoma tissues.

9. The medical agent according to claim 8, wherein the effector agent is provided with positron-imaging radionuclides, preferably F-18, Br-75, Br-76 and I-124; therapeutic radionuclides, preferably Y-90, I-131, In-114m, Re-186, Re-188, Cu-67, Sm-157, Lu-177, Bi-212, Bi-213, At-211, Ra-223, gamma-imaging radionuclides, preferably Tc99m, In-111, I-123 and I-125, beta-radiation emitters, preferably scandium-46, scandium-47, scandium-48, copper-67, gallium-72, gallium-73, yttrium-90, ruthenium-97, palladium-100, rhodium-101, palladium-109, samarium-153, lutetium-177, rhenium-186, rhenium-188, rhenium-189, gold-198, radium-212, and lead-212, gamma emitters, preferably iodine-131 and indium-m114 and positron emitters, preferably gallium-68 and zirconium-89.

10. The medical agent according to claim 9, wherein the effector agent comprises aryl halides and vinyl halides for radionuclides of halogens, N_2S_2 and N_3S chelates for Tc and Re radionuclides, amino-carboxy derivatives, preferably EDTA and DTPA or derivatives thereof, and cyclic amines, preferably NOTA, DOTA and TETA, and derivatives thereof, for In, Y, Pb, Bi, Cu, Sm

and Lu radionuclides, or any other radionuclide capable of forming a complex with said chelates.

11. The medical agent according to claim 10, wherein the effector agent comprises DOTA and is provided with Y-90 or Lu-177 for therapeutic application or In-111 for diagnostic purposes.

12. The medical agent according to any one of the preceding claims, wherein the biotin derivative is chosen from the group consisting of norbiotin, homobiotin, oxybiotin, iminobiotin, destibiotin, diaminobiotin, biotin sulfoxide, and biotin sulfone, or derivatives, preferably norbiotin or homobiotin.

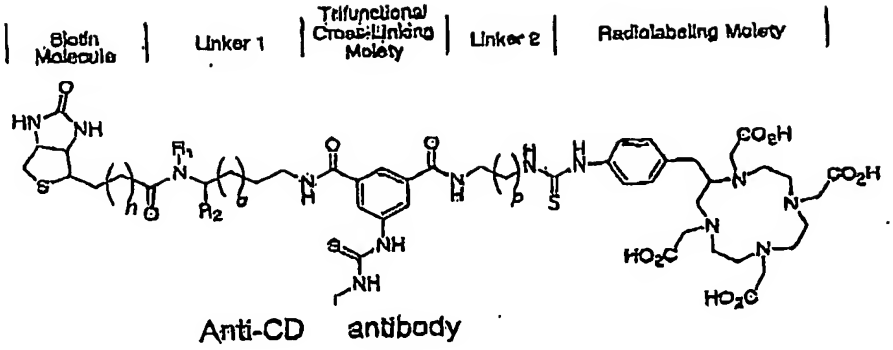
13. The medical agent according to any one of the preceding claims, wherein the biotinamide amine substituents are $-CH_2OH$ or $-CO_2H$ and the substituents adjacent to the biotin amine are $-CH_3$ or $-CH_2OH$.

14. The medical agent according to any one of the preceding claims, wherein the anti-CD20 antibody has been covalently bound to the reagent, optionally via the linker 3, through a reaction of a group of active esters consisting of N-hydroxysuccinimide esters, sulfo-N-hydroxysuccinimide esters, and phenolic esters; aryl and alkyl imidates; alkyl or aryl isocyanates or isothiocyanates, with amino groups on the anti-CD20 antibody; or a reaction of maleimides or alphahaloamides with sulfhydryl groups on the anti-CD20 antibody; or a reaction of aryl or alkylhydrazines or alkyl or aryl-hydroxylamines with aldehyde or ketone groups naturally occurring or synthetically produced on the anti-CD20 antibody.

15. The medical agent according to any one of the preceding claims, wherein the linker 2 is excluded.

16. The medical agent according to claims 1-15,

wherein it is



wherein the anti-CD20 antibody preferably is rituximab, wherein n is 2-4, preferably 3, o is 1-6, preferably 3, p is 1-6, preferably 3; R_2 is $-CH_2OH$ or $-CO_2H$; and R_1 is $-CH_3$, $-CH_2OH$ or $-H$.

17. The medical agent according to claim 16, wherein it is 3-(13'-thioureabenzyl-(DOTA)trioxadiamine-1-(13''-biotin-Asp-OH)trioxamine-5-isothio-cyanato-aminoisophtalate-ibritomumab, 3-(13'-thioureabenzyl-(DOTA)trioxadiamine-1-(13''-biotin-Asp-OH)trioxamine-5-isothio-cyanato-aminoisophtalate-rituximab, or 1-Isocyanato-3-((1S'-(N-Biotinyl)-β-L-Asparty1)-4',7',10'-Trioxa-penta-Decanylamino)-1-((13-(Benzylthiourea-CHX-A'')-4,7,10-Trioxatridecanediamine)-Aminosiophtalate-rituximab, preferably 3-(13'-thioureabenzyl-(DOTA)trioxadiamine-1-(13''-biotin-Asp-OH)trioxamine-5-isothio-cyanato-aminoisophtalate-rituximab.

18. The medical agent according to any one of the preceding claims, wherein it further comprises physiologically acceptable additives, preferably an ammonium acetate solution.

19. A medical agent according to any one of the preceding claims, with the proviso that said reagent/-reagents is/are covalently bound to the ant-CD20 antibody without the linker 3.

20. A kit for extracorporeal elimination or at least reduction of the concentration of a non-tissue bound therapeutic or diagnostic medical agent as defined in any one of claims 1-19 in the plasma or whole blood of a mammalian host, wherein said medical agent previously has been introduced into a mammalian host and kept therein for a certain time in order to be concentrated to the specific tissue or cells by being attached thereto, said kit comprising

a) the medical agent, and

b) an extracorporeal device comprising an immobilised receptor to which a biotin molecule adheres.

21. Use of a medical agent according to any one of claims 1-19 or the kit according to claim 20 for the treatment of lymphoma, preferably non-Hodgkin's lymphoma.